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14. ABSTRACT

The purpose of this contract is to carry out emerging infectious disease surveillance in Kenya. Specific areas in which work is performed include respiratory illness surveillance (particularly influenza), acute febrile illness surveillance, malaria resistance surveillance, diarrhea etiology and antimicrobial resistance surveillance, sexually transmitted illness surveillance, and capacity building. KEMRI maintained surveillance sites in both Ministry of Health and now Kenyan Defense Forces clinics and hospitals throughout Kenya. KEMRI operated reference laboratories for this work in Nairobi, Kericho, and Kisumu, including the National Influenza Center (NIC), the arbovirus reference laboratory, the antimalarial resistance laboratory, entomology facilities, the Center of Excellence in Microscopy, the microbiology reference laboratory. Capacity development projects include continuation of a laboratory and medical maintenance student attachment program and a safety training program. The program was able to characterize respiratory viruses causing influenza-like illness in Kenya, determine etiologies of diarrheal illnesses and the antimicrobial resistance patterns of bacterial causes, determine the etiologies of sexually transmitted infections and acute febrile illnesses in military and civilian populations, and establish the pattern of antimalarial resistance across Kenya. Outbreak investigation and response continues. AFI expanded into regions around Somalia.

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INTRODUCTION:

KEMRI supports USAMRU-K's establishment of an emerging infectious disease surveillance network by providing contract personnel, laboratory and administrative facilities, capacity development capabilities for contracted personnel and partner organizations, regulatory oversight, and other required functions for the performance of infectious disease surveillance and research. The areas of research/surveillance performed are categorized by the pillars as defined by the US Department of Defense's Armed Forces Health Surveillance Center Department of Global Emerging Infectious Disease Surveillance and Response (DoD-GEIS). These pillars include respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

BODY: For clarity's sake, this report will be divided by DoD-GEIS pillar.

Respiratory Illness:

Global influenza surveillance is an important scientific activity since it aids in the detection of viral antigenic shift and drift which are responsible for pandemics and epidemics respectively. Our initial results of the surveillance network have revealed a distinct genetic variant of H3N2 influenza virus which emerged in the poor, crowded, informal housing settlement of Kibera. This strongly suggests that a future highly pathogenic and dangerous strain could emerge in Africa and not only South East Asia and supports our continued surveillance work and capacity building. We have also begun to examine genetically and temporally how the strains obtained in Kenya differ from the vaccine strains and other global circulating strains especially those in other Sub Saharan African countries. Comparisons to other Sub Saharan African countries is difficult except for South Africa because publicly available genetic sequences and raw data for influenza are not readily available. Our tentative conclusions from a recent analysis are that over two full years of surveillance strongly suggests that influenza is seasonal with most infections centered around the cool months of June through September in most locations, but possibly not along the warmer coast. The fact that 15-20% of the ILI specimens collected have been positive for influenza indicates that influenza is causing a significant portion of the upper respiratory disease in Kenya. The detectionH1N1 strain drift to A / Brisbane / 59 / 2007-like viruses in African strains in 2007 one half year prior to it being reported elsewhere reinforces importance of influenza surveillance in Africa. Small clade differences between Kenya and other Sub-Saharan African regions suggests possible regional differences within Africa. The GEIS Program in East and Central Africa has made a significant positive impact on the local public health communities where we operate in the response to the influenza A (H1N1) pandemic in 2009 and 2010.

Between 1st October and 31st December 2009, the Kenya National Influenza centre analyzed a total of 1332 respiratory samples for viral causes of respiratory disease. These included 1013 samples from the regular USAMRU-K sentinel surveillance network and 319 pandemic influenza A (H1N1) strain investigation samples from Kenya. Of the 1013 regular USAMRU-K sentinel surveillance samples tested since October 2009, 37.9% tested positive for influenza viruses. In total, 360 samples tested positive for influenza. Majority (72.2%) of those testing positive for influenza were type A viruses. When the influenza A viruses were subtyped using PCR, 34.6% did not subtype. This does not necessarily indicate that these were unsubtypable. The Ct values of the PCR typing assays in all these cases were high. It has been shown that for specimens showing high Ct values (i.e. those with low viral loads), PCR subtyping assays usually do not work well and this is likely the reason for the failure of these to subtype. Among the 170 specimens that subtyped, the predominant subtype (at 81.8%) was pandemic influenza A H1N1 strain followed by A/Brisbane/10/2007 (H3N2)-like (14.1%,). As seen elsewhere in the world, the 2009 Pandemic influenza A H1N1 became the predominating strain despite having been introduced into Kenya barely six months prior. Only

4% of the samples that tested positive for influenza A using PCR assay were A/Brisbane/59/2007 (H1N1)-like. All the influenza B positive samples were B/Florida/4/2006-like. Eighty five influenza viruses were isolated from the one hundred and twenty two inoculations representing a 70% isolation rate. All the isolates obtained were pandemic influenza A H1N1 strain Twenty four patients (representing 6.6% of influenza positives) had dual infections of influenza A and B. In addition, out of the 170 influenza A positive subtyped samples, 2.4% were dual infections of pandemic influenza A H1N1 and Influenza B/Florida/4/2006-like. Furthermore, only one of positive samples was a dual infection of pandemic influenza A H1N1 and seasonal influenza A/Brisbane/10/2007 (H3N2)-like and one for pandemic influenza A H1N1and seasonal influenza A/Brisbane/59/2007 (H1N1)-like. Pandemic Influenza Outbreak Investigations for Kenya Six months after being introduced into Kenya, the novel 2009 pandemic influenza A H1N1 is the predominant strain in Kenya. In the 1st quarter FY 10, 319 samples were sent to the NIC from across the country for diagnostic testing for the presence of the novel 2009 pandemic influenza A H1N1. Out of these 206 samples representing 64.5% tested positive for influenza viruses. Among the 206 that tested positive for influenza, majority (66.5%) were the novel 2009 pandemic influenza A H1N1 strain. 18% of the suspected cases had influenza B and not the pandemic strain. Only 4.8% of the suspected cases had seasonal influenza A infection. Out of the 167 samples that tested positive for influenza A, 13.8% did not subtype. Representing by 11.2% of all the positive samples, multiple infections of influenza types and subtypes were seen during the period under review. Of these, 18 (representing 78.3%) were dual infection of pandemic influenza A H1N1 and Influenza B/Florida/4/2006-like, with influenza A H3N2 & influenza B at 8.7%, and pandemic H1N1 & seasonal H1N1 at 4.3%. Likewise, pandemic H1N1 & seasonal H3N2 dual infections were present at 4.3% of the samples. A triple infection of pandemic influenza A H1N1, Influenza B & seasonal influenza A (H3N2) were also noted at 4.3% of the infections.

Between 1st January and 31st March 2010, the Kenya National Influenza centre analyzed a total of 880 respiratory samples for viral causes of respiratory disease. These included 844 samples from the regular USAMRU-K sentinel surveillance network and 36 pandemic influenza A (H1N1) strain investigation samples from Kenya. Of the 844 regular USAMRU-K sentinel surveillance samples tested since January 2010; all the specimens were screened for influenza by PCR. 13.5% tested positive for influenza viruses. Majority (77%) of those testing positive for influenza were type A viruses. When the influenza A viruses were subtyped using PCR, 79.5% did not subtype. This does not indicate that these were unsubtypable. The Ct values of the PCR typing assays in all these cases were high. Among the 18 specimens that subtyped, the predominant subtype (at 88.9%) was pandemic influenza A H1N1 strain. Seasonal influenza A H1N1 was not detected in Kenya by our surveillance network in the months of January to March. As seen elsewhere in the world, the 2009 Pandemic influenza A H1N1 is the predominating strain in Kenya. All the influenza B positive samples were B/Brisbane/60/2008-like. During the second quarter, we isolated thirty influenza B/Brisbane/60/2008-like viruses from samples collected in the first quarter. We also isolated eight A/Brisbane/59/2007 (H1N1)-like and three A/Brisbane/10/2007 (H3N2) like viruses. One patient had dual infections of seasonal influenza A and B and one patient had a triple infection of seasonal influenza A & B and pandemic H1N1. In addition out of the 170 influenza A positive subtyped samples, 2.4% were dual infections of pandemic influenza A H1N1 and Influenza B/Florida/4/2006-like. Furthermore, only one of the positive samples was a dual infection of pandemic influenza A H1N1 and seasonal influenza A/Brisbane/10/2007 (H3N2)-like and one for pandemic influenza A H1N1and seasonal influenza A/Brisbane/59/2007 (H1N1)-like. Between January and March 2010, we received and 36 outbreak samples for pandemic H1N1 investigation. Nine of these tested positive for pandemic H1N1. The 2009 pandemic influenza A H1N1 remains the predominant influenza A strain in Kenya and this co-circulated mainly with influenza B. This co-circulation trend has also been reported in other parts of the world. Overall, the pandemic flu activities have drastically diminished since

December 2009. In the 1st quarter FY 10, 319 samples were sent to the NIC from across the country for diagnostic testing for the presence of the novel 2009 pandemic influenza A H1N1. Out of these 206 samples representing 64.5% tested positive for influenza viruses. Among the 206 that tested positive for influenza, majority (66.5%) were the novel 2009 pandemic influenza A H1N1 strain. 18% of the suspected cases had influenza B and not the pandemic strain. Only 4.8% of the suspected cases had seasonal influenza A infection. Out of the 167 samples that tested positive for influenza A, 13.8% did not subtype. Between 1st April and 30th June 2010, the Kenya National Influenza centre analyzed a total of 669 respiratory samples for viral causes of respiratory disease. These included 660 samples from the regular GEIS sentinel surveillance network and 9 outbreak investigation specimens for detection of the pandemic influenza A (H1N1) strain. None of these outbreak samples tested positive for influenza. All the 660 regular GEIS influenza surveillance specimens collected in this quarter were screened for influenza by real time RT-PCR. 10.1% tested positive for influenza viruse. For those samples that were positive for influenza, majority (91%) were influenza type A viruses. When the influenza A positive sample were assayed for the subtyped using PCR, 24.6% did not subtype. This does not indicate that these were unsubtypable. The Ct values of the PCR typing assays in all these cases were high. Among the 46 specimens that subtyped, the predominant subtype was the seasonal H3N2 strain at 73.8%. Only one pandemic H1N1 was typed from sentinel surveillance samples. Influenza A/H3N2 was the predominant strain in circulation in Kenya during the 3rd quarter. During the third quarter, we isolated 122 disparate viruses by culture. Thirteen of these were influenza A H3N2 and one was Influenza B/Brisbane/60/2008-like virus. Overall 11.5% of the isolates were influenza viruses. While the non-influenza viruses isolated were 108 representing 88.5% of the isolates. These consisted of 28.7% RSV, 45% parainfluenza predominantly type 1 and 3, adenovirus (13.9%) and enteroviruses (9%). Single infecting agents were recovered from most specimens. However, there were dual and multiple infections of influenza A/H3N2 with parainfluenza's even among the non-influenza viruses, where coinfections were also observed mainly as parainfluenza with adenovirus, RSV or enteroviruses. These coinfections made only 10% of total isolates. Between April and June 2010, we received 9 outbreak samples for pandemic H1N1 investigation. None of them tested positive for the pandemic H1N1.This is consistent with observations from other parts of the world where the pandemic 2009 virus activity has general remained low.

Between July 1st and 30th September 2010, the Kenya National Influenza centre analyzed a total of 576 respiratory samples for viral causes of respiratory disease. These were all samples collected from the regular USAMRU-K sentinel surveillance network. All the specimens collected in this quarter were screened for influenza
by PCR. 13.2% (76/576) tested positive for influenza viruses. For those samples that were positive for influenza majority (97.4%) (74/76) tested positive for influenza type A virus. Out of the 74 influenza A the predominant subtype at 96% (71/74) was the seasonal H3N2 strain. Only three pandemic H1N1 cases were reported from sentinel surveillance samples. Influenza A/H3N2 is still the predominating strain in Kenya at the moment. During this quarter, we isolated 72 viruses by culture. Thirty one of these were influenza A type H3N2 and one was Influenza B/Brisbane like. 14 rt-PCR positive influenza turned out to be negative after culturing, while 26 are yet to be put in cultures. Fourty (7%) non-influenza viruses were isolated. These consisted of 50% (20/40) adenovirus, 30% (12/40) parainfluenzaviruses, 20% (5/40) RSV and 7.5% (3/40) enteroviruses. We recovered single infecting agents from most specimens. However, we also observed a single dual infection of influenza A/H3N2 with parainfluenza 2, another dual infection of adenovirus plus enteroviruses and a solitary case of triple infection with parainfluenza 1, 2 and 3.

Acute Febrile Illness:

Human Surveillance: 1275 cumulative samples have been received for Acute febrile illness (AFI) study from seven hospitals: Kisii, New Nyanza and Kondele in Western Kenya, Marigat in Rift Valley, Malindi in Coastal Province and Iftin in North Eastern Province. From these, nucleic acid has been extracted from 1084 samples, of which 780 have been evaluated for Brucellosis, 780 for Leptosprosis, 984 for Salmonellosis, 1054 for rickettsioses, 1054 for malaria, 984 for Dengue, 182 for measles and 195 for upper respiratory viruses. Of note is a 15% prevalence rate of malaria detected from samples that are RDT negative by PCR. We are investigating whether this RDT failure rate represents starins of P. falciparum without HRP-2 (the target antigen for RDTs) or reflect the low sensitivity of RDT compared to PCR. Invasive Salmonella were identified in 7% of the children recruited. Rickettsia was identified in 0.3% of the specimens and Brucellosis in 0.2%. All brucella positive samples came from a hospital that serves the nomadic population. Leptospirosis and Dengue have not been identified in any of the surveillance sites. Between 2nd and 3rd quarter, the following viral infections were identified in nasal pharyngeal swabs: 6% human metapneumonia, 2% adeno viruses. 2% parainfluenza 3%, 34% parainfluenza 1, 8% rhino virus, 2% Corona virus OC 43, 2% RSV B and 2% influenza A.

Somalia Ecosystem: In this year, the AFI human surveillance program expanded to include sites similar to Somalia in their ecosystem. Somalia is a country of interest to the Kenya governments as well as to US military. If handled well, the surveillance has potential to boost the US image among the Kenyan Somalis and therefore complement the AFRICOM mission. Kenya-Somalia border is porous and as such, the surveillance for Fevers of Unknown Origin (FUO) on the Kenyan side of the border offers a real possibility to identify not only the natural disease threats, but also those that have the potential to be exploited as biothreat. Limited epidemiological data, blood and nasal pharyngeal swabs are collected from patients with FUO. Collected specimens are aliquoted and stored at the sites in liquid nitrogen dry shippers and then sent via an overnight Courier to central WRP/KEMRI laboratory in Kisumu for inventory, storage and diagnostic testing. Re-charged LN dry shippers are then sent back to the sites for the next round of sampling. The AFI pathogens of interest include malaria, dengue, measles, rickettsia, salmonella, brucellosis,leptospirosis, upper respiratory tract viruses, Q-fever, Relapsing fever, Hanta viruses and other arboviruses. Of the 327 specimens analyzed, 28 had malaria (27 due to P. falciparum and one P. vivax) and 3 had bacteremia. Following sequencing of the bacteremic samples with 16S RNA primers, one of the samples was found to have a Burkholderia sp (see attached sequences). Members of the Burkholderia cepacia complex (Bcc), have been found in many natural environments, but recently there has been emergence of many Bcc infections.

Arbovirology:

Between July and September field visits were made to 5 of the surveillance regions for vector collection. A total of 279,726 mosquitoes and 7,000 ticks were collected. 106,534 (5913 pools) mosquitoes and 6,000 ticks were identified in total. A total of 47 isolates were obtained in the fourth quarter with 9 identified to the species level 1 identified to the family level only and 37 still unidentified.

Region	Total inoculated	Total # of isolates	Virus identity		
			Family	Species	Unidentified
MAGADI	prior	7			7
TURKANA	prior	1			1
GARRISA	236	18	Bunyavir idae (1)	West Nile (5) Pongola (1) Ndumu (2)	9
TANA DELTA	1266	18		1 (Ndumu)	17
Naivasha	2				
RABAI	61	3			3
BUDALANGI	462	In progress			
Totals	**2027**	**47**	**1**	**9**	**37**

Leishmania:

Results: Data to date

Site	Approx No of sandflies collected	No identified	Phlebotomus	Sergentomyia	PCR tested	No +ve	Real time	Infection rates
Isiolo	**18,000**	**1973**	1172 (59%)	800 (41%)	5153	6	3	0.058219
Wajir	5,000	1166	530 (46%)	636 (54%	587	0	0	
Garissa	17,000	2163	89 (4%)	2074 (96%)	1417	2	0	0
Lamu	2000	592	14 (2%)	362 (80%)	259	6	1	0.3861
West Pokot	1000	168	82 (49%)	86 (51%)	0	0	0	0
Turmi, Ethiopia	200	21	0	21 (100%)	0	N/A		
Gilgil	1200	150	146 (97%)	4 (3%)	342	6		
Tanzania	800	120	4 (3%)	116 (97%)	195	3		
Total	45200	6353	2037	4100	7611	23	4	

Infection rates calculated as No of real time PCR pools/total no tested*100.
PCR tested = conventional PCR

Outbreaks:

Between April and June, field visits were made to six sites for vector collection following reports of potential RVF outbreak due to prolonged heavy rains across the country. This RVF alert surveillance covered 6 regions of the country (Magadi, Turkana, Garissa, Naivasha, East Pokot, Kitale and Tana Delta). A total of 152268 mosquitoes and 15,000 ticks were collected in addition to 560 samples from sick livestock.

The laboratory identified a Dengue-1 outbreak in Massawa Province of Eritrea based on IgM ELISA and PCR testing of 26 samples sent to the laboratory.

The laboratory diagnosed a Rift Valley Fever case from Southern Sudan and a complex Flavivirus positive patient from Wajir, Kenya with Yellow fever, Dengue and West Nile seropositivity by IgG and IgM ELISA. This is still awaiting confirmation. 15/17 other samples received by the lab were negative for the standard panel of arboviruses and hemorrhagic

fevers: IgM ELISA (Yellow Fever, Dengue, West Nile, Chikungunya, Rift Valley Fever, Crimean Congo Hemorrhagic Fever.)

Malaria:

P. falciparum drug resistance, conferred largely by mutations, is expected to continue evolving in Kenya. Tracking in vitro Pf malaria drug resistance patterns and molecular mutations can herald new patterns useful in selecting effective malaria drugs.

Molecular results Pfcrt Gene October 2009-June 2010			
Pfmdr1 SNPs	**Codon 76**		
Study Site (n)	**W**	**M**	**W/M**
KDH (99)			
1st Quarter (64)	0	35/64 (55%)	29/64 (45%)
2nd Quarter (35)	1/23 (4%)	5/23 (22%)	17/23 (74%)
3rd Quarter (69)	**23/59 (39%)**	**29/59 (49%)**	**7/59(12%)**
Total %	24/146 (16.4%)	69/146 (47.3%)	53/146 (36.3%)
KCH (19)			
1st Quarter (5)	0	0	3/3 (100%)
2nd Quarter (14)	0	3/11 (27%)	8/11 (73%)
3rd Quarter (25)	**7/17 (41%)**	**9/17 (53%)**	**1/17 (6%)**
Total %	7/31 (22.5%)	12/31 (38.7%)	12/31 (38.7%)
KSI (34)			
1st Quarter (19)	0	6/10 (60%)	4/10 (40%)
2nd Quarter(15)	0	3/12 (25%)	9/12 (75%)
3rd Quarter (31)	**6/25 (24%)**	**19/25 (76%)**	**0**
Total %	6/47 (12.8%)	28/47 (59.5%)	13/47 (27.7%)
KEY			
M	Mutant		
W	Wild		
W/M	Mixed Infection		

Kisumu East District Hospital in vitro antimalarial drug resistance profiles, 2008-2010

Drug	**Doxycycline**			**Amodiaquine**			**Artemisinin**			**Quinine**			**Mefloquine**			**Chloroquine**		
Study Year	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
N. of samples	38	46	27	51	44	34	42	44	41	45	43	36	50	45	38	49	39	41
Median	12141	9805	11507	5.16	2.84	1.901	2.55	2.16	2.676	72.31	37	52.13	7.584	7.524	6.703	29.7	22.2	11.73
Mean	16897	16712	18206	9.354	5.58	6.439	2.99	2.52	1.603	104.8	58.6	96.38	18.88	19.88	17.88	42.7	36.4	23.78
Standard Deviation	16298	17239	24841	11.87	7.36	10.93	2.28	1.61	3.031	88.53	83.5	123	24.03	29.01	29.01	46.2	48.7	29.6
% Sensitive	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	93	98	91	56	67	55	71	74	82
% Resistant	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7	2	9	44	33	45	29	26	18

KEY: **KEY**: 50% inhibition concentration in ng/ml expressed as Means and medians; NA = Cut off point for Resistance/sensitivity not available

Kisii District Hospital in vitro antimalarial drug resistance profiles, 2008-2010

Drug	**Doxycycline**			**Amodiaquine**			**Artemisinin**			**Quinine**			**Mefloquine**			**Chloroquine**		
Study Year	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
N. of samples	11	12	20	12	12	23	13	12	20	14	12	21	12	13	25	12	13	21
Median	9589	6541	7574	2.916	2.88	1.916	3.82	2.12	2.72	60.88	25.9	27.9	7.497	5.726	9.703	41.5	23.2	14.82
Mean	18230	15006	12601	3.52	3.59	19.81	4.82	3.31	6.47	55.86	26.9	124	7.32	38.36	19.19	44.5	43.2	53.42
Standard Deviation	24331	19567	16346	2.41	2.45	59	4.3	3.53	11.96	32.87	19.6	233.3	4.314	113.1	26.77	24.7	56.9	90.2
% Sensitive	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	100	100	86	67	77	52	58	77	76
% Resistant	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	14	33	23	48	42	23	24

KEY: **KEY**: 50% inhibition concentration in ng/ml expressed as Means and medians; NA = Cut off point for Resistance/sensitivity not available

Kericho District Hospital in vitro antimalarial drug resistance profiles, 2008 & 2010

Drug	Doxycycline			Amodiaquine			Artemisinin			Quinine			Mefloquine			Chloroquine		
Study Year	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
N. of samples	9		14	13		13	12		10	10		10	15		13	12		10
Median	129339	TU	10441	3.436	TU	2.398	5.05	TU	2.75	64.27	TU	64.27	9.011	TU	35.33	12.9	TU	22.25
Mean	197141	TU	16198	4.569	TU	8.617	6.18	TU	8.276	59.77	TU	59.77	15.22	TU	47.93	29.6	TU	37.67
Standard Deviation	233036	TU	23298	3.658	TU	17.28	4.68	TU	10.3	32.22	TU	32.72	19.28	TU	45	39.2	TU	40.75
% Sensitive	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	100	TU	73	53	TU	30	83	TU	70
% Resistant	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	TU	27	47	TU	70	17	TU	30

KEY: 50% inhibition concentration in ng/ml expressed as Means and medians; NA = Cut off point for Resistance/sensitivity not available; TU = Testing Underway

Results provide baseline in vitro drug sensitivity profiles for six antimalarial drugs. No day 7 malaria positive was diagnosed during the three year study period, which is in tandem with the observed stable artemisinin IC_{50}s (Table 1, 2 & 3). Chloroquine IC_{50}s showed greater than 60% isolates "sensitive" (< 45.5 ng/ml), maybe secondary to reduced drug pressure. Since there is no established threshold value, artemisinin IC50s here is a baseline future comparator for this study.

In vitro drug sensitivity: In the 1st quarter of FY10, 92 Pf specimens were collected from three sites in western Kenya. All were tested for susceptibility against 10 antimalarial drugs using the MSF (SYBR green I) assay. All samples were tested along with two index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. Screening drugs included at least chloroquine (CQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), primaquine (PQ) and doxycycline (DX). Drug sensitivity profiles were expressed as 50% inhibition concentration (IC50s). The 92 field specimens were screened (49 using immediate ex vivo culture and 11 following culture-adaptation), along with the reference clones (W2 & D6). AT and LU IC50 medians were 0.9903 ng/ml (n=43) and 14.28 ng/ml (n=49) respectively. This data provides baselines for continued tracking of their profiles. AT and LU are the two components of the first-line therapy for Pf malaria in Kenya. In vitro drug testing of Pf isolates is on course in tandem with continued recruitment.

During 2QFY10, 121 Pf specimens were collected from three sites in western Kenya bringing the FY10 total to 213 samples. All were tested for susceptibility against 11 antimalarial drugs using the MSF (SYBR green I) assay alongside two index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. Screening drugs included at least chloroquine (CQ), mefloquine (MQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), primaquine (PQ) and doxycycline (DX). Drug sensitivity profiles were expressed as 50% inhibition concentration (IC50s). The 121 field specimens were screened (21 using immediate ex vivo culture and 20 following culture-adaptation), along with the reference clones (W2 & D6). This data provides baselines for continued tracking of their profiles. AT and LU are the two components of the first-line therapy for Pf malaria in Kenya. In vitro drug testing of Pf isolates is on course in tandem with continued recruitment.

During 3Q, 141 Pf specimens were collected from three sites in western Kenya bringing the FY10 total to 354 samples. Most of these samples were tested for susceptibility against 11 antimalarial drugs using the MSF (SYBR green I) assay alongside two index Pf clones

[chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. Screening drugs included at least chloroquine (CQ), mefloquine (MQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), primaquine (PQ) and doxycycline (DX). Drug sensitivity profiles were expressed as 50% inhibition concentration (IC50s). The 354 field specimens were screened (25 using immediate ex vivo culture and 18 following culture-adaptation), along with the reference clones (W2 & D6). This data provides baselines for continued tracking of their profiles. Findings of drug response profiles across 3 study sites, for 6 drugs are summarized on Tables 3, 4 and 5. AT
and LU are the two components of the first-line therapy for Pf malaria in Kenya. In vitro drug testing of Pf isolates is on course in tandem with continued recruitment.

During 4QFY10, 92 Pf specimens were collected from 4 sites, (3 in western Kenya and 1 in Eastern Kenya) bringing the FY10 total to 446 samples. Most of these samples were tested for susceptibility against 11 antimalarial drugs using the MSF (SYBR green I) assay alongside two index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. Screening drugs included at least chloroquine (CQ), mefloquine (MQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), primaquine (PQ) and doxycycline (DX). Drug sensitivity profiles were expressed as 50% inhibition concentration (IC50s). The 92 field specimens collected, 28 using
immediate ex vivo culture and 18 following culture-adaptation are frozen pending repair/replacement of the fluorometer. This data and that of previous quarters is to provide baselines for continued tracking of their profiles. Findings of drug response profiles across 3 study sites, for 6 drugs are summarized on Tables 3, 4 and 5. AT and LU are the two components of the firstline therapy for Pf malaria in Kenya. In vitro drug testing of Pf isolates is on course in tandem with continued recruitment. Molecular assays: During quarter 4, PCR was done on 81 samples for mutation analysis of the Pfmdr1 gene. The IC50 results were compared to the mutation profile at codon 86. Cumulatively, we still observe that PfMDR1 mutation rates were lower for codon 86 as compared to the previous year (Table 2). We also continue to observe that codon 86 mutant genotypes have lower IC50s in vitro (Figure 1). We have observed a number of mutations in the PfATPase gene; however, none of the observed mutations have any effect on artemisinin drug susceptibility (Figure 2). There is modest Pfcrt mutation profile

The Malaria Diagnostics Center (MDC) was established in Kisumu, Kenya in 2003. To date, the primary mission has been to provide malaria microscopy training (Ohrt, et al, 2007). In 1QFY10, 95 volunteers were screened. 20 subjects were enrolled. An average of 269 standardized malaria blood film slides and 15 frozen blood aliquots prepared from each subject. 171 volunteers screened. 38 subjects enrolled (37 *Plasmodium*-positive, 1 *Plasmodium*-negative). An average of 266 standardized malaria blood film slides and 14 frozen blood aliquots prepared from each subject

Enterics:

Acute gastroenteritis, including diarrhea, is a debilitating illness that can rapidly compromise an individual's regular daily functions. The current protocol is designed to strengthen our surveillance capabilities in Kenya and the AFRICOM area of responsibility for the detection of key microbial enteric pathogens. The bacterial identification includes but is not limited to *Enterotoxigenic E. coli* (ETEC), *enterohemorrhagic E. coli* (EHEC), *Enteroaggregative E. coli* (EAEC), *Yersinia enterocolitica*, *Vibrio cholerae*, *Campylobacter jejuni*, *Salmonella* spp., and *Shigella* spp. Identification and antibiotic susceptibility testing is performed on the Siemens Microscan system. Parasitic pathogens are currently being diagnosed via microscopy and viral (rotavirus) causes are identified by enzyme immunoassays (EIA). The immediate benefit of this study is having a well defined sample of the spectrum of

diarrheal pathogens in sub-Saharan Africa as the number of US military personnel deployed to Africa significantly increases.

Total 192 specimens were received and processed during the period of 1 January 2010 – 31 March 2010. Of 192 specimens, 102 were unknown specimens and 90 were controls. The detection of rotavirus and protozoan was not performed due to the shortage of the Rotavirus clone kit and Triage kit. Serotyping *Salmonella* and *Shigella* were not performed for all received samples because of a shortage of the specific reagents. We have received Rotavirus clone kit and anti-sera of *Salmonella* and *Shigella* recently and should receive the Triage kit soon. Untested samples will be tested as soon as the Triage kit is received. Of 192 specimens, 20 had bacterial isolates, but not included *E coli* pathogenic isolates.

A total of 133 samples were received and processed during the 3rd quarter of FY10 of which 71 were cases and 62 were controls. Shigella spp was the most common bacterial pathogen and rotavirus the most common overall pathogen. Giardia lamblia was the most common parasite.

A total of 111 samples were received and processed during the 4th quarter FY10 of which 55 were cases and 56 were controls. Shigella spp was the most common bacterial pathogen and Giardia lamblia was the most common parasite.

A total of 589 stool samples have been processed since 29 Sept 2009. A wide range of enteric pathogens have been detected using the diagnostic methodologies in the lab.

Sexually Transmitted Infections:

The prevalence and drug resistance profiles of chlamydia and gonorrhea are largely unknown in Kenya. This project begins to address these questions, which are likely relevant to all of East Africa, and of importance to the DoD and Kenya MoH.

Out of a total of 159 cases from the start of the screening, there have been 36 cases of *Chlamydia trachomatis* and 68 cases of *Neisseria gonorrhoeae* as determined by the OnSite Rapid diagnostic tests for the two pathogens. There have been 25 cases of co-infection with both pathogens.

Capacity Development:

The KWHDSS is a research project designed to generate social health and demographic data which can be used in disease surveillance, outbreak monitoring and response, and improving public health infrastructure throughout Western Kenya. The KWHDSS has been set up to better monitor demographic changes and disease prevalence in the area by integrating household-based and health-facility-based data, focusing on health conditions of the population. Data from the KWHDSS will be analyzed with data from health facilities and other sources to produce robust estimates that give a comprehensive picture of the health condition of the community. The longitudinal nature of the KWHDSS will also help in better understanding health in the context of time and space. The district has a total Population of 139,700 people of all age groups. The population per sub location within the district ranges from 1,422, for the least populated to 8,615 for the most populated. The male to female ration in the District is 0.92: 1 (47.7%: 52.3%). Age groups under 1 year comprise of 3.7% of the total population. The percentage of ages 1-4 is 12.1% while the percentage of ages 1-4 is 40.5%. The majority of the population group is aged above 18 years at 43.7% of the whole population. The population density of Kisumu west is approximately 399 person's sq/km. However, this varies considerably between the two division and different locations.

KEY RESEARCH ACCOMPLISHMENTS:

Characterization of etiologies of influenza-like illness in Kenya

Identification of circulating strains of Influenza virus in Kenya

Characterization of selected viral, bacterial, and rickettsial etiologies of febrile illness in Kenya

Continued elucidation and tracking changes in antimalarial resistance patterns in Kenya

Ongoing characterization of etiologies of diarrheal illnesses in Kenya

Human and infrastructure capacity development programs

REPORTABLE OUTCOMES: See references.

CONCLUSION:

KEMRI provides critical support to USAMRU-K's emerging infectious disease surveillance program in Kenya. Without KEMRI, USAMRU-K would not be able to execute its mission. KEMRI provides the legal and regulatory framework, personnel, and laboratory structure necessary to carry out scientific work. The organizations exist in partnership, with USAMRU-K working fully under the KEMRI umbrella in Kenya. Together, we have made great strides in establishing surveillance capabilities in the areas of respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

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